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ORIGINAL ARTICLE

Fabrication and characterization of polycaprolactone and tricalcium phosphate composites for tissue engineering applications

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KEYWORDS

β-tricalcium phosphate;
biocomposites;
biodegradable;
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Abstract *Background/purpose:* β-Tricalcium phosphate (β-TCP) is an osteoconductive material which has been used for clinical purposes for several years, as is polycaprolactone (PCL), which has already been approved for a number of medical and drug delivery devices. In this study we have incorporated various concentrations of β-TCP into PCL with the aim of developing an injectable, mechanically strong, and biodegradable material which can be used for medical purposes without organic solvents.

Materials and methods: This study assesses the physical and chemical properties of this material, evaluates the *in vitro* bioactivity of the PCL/β-TCP composites, and analyzes cell proliferation and osteogenic differentiation when using human bone marrow mesenchymal stem cells (hBMSCs).

Results: The results show that weight losses of approximately 5.3%, 12.1%, 18.6%, and 25.2% were observed for the TCP0, TCP10, TCP30, and TCP50 composites after immersion in simulated body fluid for 12 weeks, respectively, indicating significant differences ($P < 0.05$). In addition, PCL/β-TCP composites tend to have lower contact angles ($47 \pm 1.5^\circ$ and $58 \pm 1.7^\circ$ for TCP50 and TCP30, respectively) than pure PCL ($85 \pm 1.3^\circ$), which are generally more hydrophilic. After 7 days, a significant (22% and 34%, respectively) increase ($P < 0.05$) in alkaline phosphatase level was measured for TCP30 and TCP50 in comparison with the pure PCL.

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Conclusion: PCL/TCP is biocompatible with hBMSCs. It not only promotes proliferation of hBMSCs but also helps to differentiate reparative hard tissue. We suggest 50% (weight) PCL-containing β -TCP biocomposites as the best choice for hard tissue repair applications.

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Introduction

Effectively and uneventfully regenerating defective hard tissue in deeper and complex bone tissue has long been a goal for clinicians, but it is a complicated issue, and progress has been slow. Materials with varying degrees of bioactivity and degradation are required so as to conform to differing clinical requirements for bone tissue engineering.¹ In previous clinical work, the most successful treatment has been the use of autologous bone grafts for bone defects. Autografting meets the requirements which are indispensable for new bone formation, namely, osteogenesis, osteoconductivity, and osteoinductivity, and it is currently considered the gold standard in this field.

Natural bone is a composite material, consisting of organic matrixes such as collagen, and extracellular matrix (ECM) linked with glycoprotein and calcium phosphate ceramics.² In order to ameliorate its relative disadvantages in regards to material degradation, we experimented with the use of β -tricalcium phosphate (β -TCP) as an additive to see how it would affect the rate of decay of polycaprolactone (PCL). β -TCP is a bioceramic material that is widely used for hard tissue repair; it has a chemical composition similar to the apatite naturally present in bone tissue, and has been applied extensively as a bone grafting material.^{3–5} Although β -TCP has various favorable characteristics that recommend its clinical use, it also has drawbacks which have proven problematic. For example, it is difficult to deliver to the required site and hard to compact adequately due to it being relatively brittle. Efforts have been made in recent years to overcome these disadvantages, leading to the use of several polymers, such as poly(L-lactic acid),⁶ PCL,⁷ poly(lactide-co-glycolide),⁸ and poly(3-hydroxybutyrate),⁹ which have the potential to improve the handling properties of β -TCP.

PCL was chosen for this study. It is a semicrystalline linear aliphatic polyester with a high degree of crystallinity and hydrophobicity. PCL has already been approved for a number of medical and drug delivery devices and is now extensively used for tissue regeneration owing to its cost-effectiveness, durability, excellent biocompatibility, and biodegradability.¹⁰ In the human body, PCL breaks down over a period of time with no side-effects; it generally takes from 6 months to 2 years to degrade *in vivo*, depending on its molecular weight.¹¹ The high mechanical strength and low degradation rate of PCL give PCL-based biomaterials several advantages for application in long-term hard tissue engineering.¹² However, PCL-based biomaterials and composites also have some shortcomings due to their slow degradation rate and lack of bioactivity, restricting their application in hard tissue engineering.¹³ To overcome these limitations,

certain modifications will need to be discovered. The most promising approach presently being explored is incorporating different polymers into other inorganic materials. The work so far in this direction has been exciting, and is considered a promising strategy for designing useful scaffolds for bone tissue engineering.^{14,15}

In the present study we have incorporated various concentrations of β -TCP into PCL, with the aim of developing an injectable, mechanically strong, and biodegradable material while avoiding the use of organic solvents. This study assesses the physical and chemical properties of this material, and evaluates the *in vitro* bioactivity of the PCL/ β -TCP composites. Cell proliferation and osteogenic differentiation are considered using human bone marrow mesenchymal stem cells (hBMSCs).

Materials and methods

Preparation of PCL/ β -TCP composites

The PCL/ β -TCP composite material used in this study was obtained by mixing reagent grade PCL (molecular weight = 43,000–50,000; Polysciences, Warrington, PA, USA) and β -TCP (Sigma-Aldrich, St. Louis, MO, USA) powder with composite weight ratios of 100:0 (TCP0), 90:10 (TCP10), 70:30 (TCP30), and 50:50 (TCP50) weight-% at 1300 g for 15 minutes using a hybrid-defoaming mixer, after which the mixture was ball-milled in ethyl alcohol using a centrifugal ball mill (S 100; Retsch, Hann, Germany) for 6 hours. The PCL/ β -TCP powder was then molded in a Teflon mold (diameter, 12 mm; height, 3 mm) and placed in an oven at 90°C for 30 minutes. The composite quantities were sufficient to fully cover each well of the 24-well plate (GeneDireX, Las Vegas, NV, USA) to a thickness of 2 mm for cell experiments.

Setting time and injectability

The setting time of the composites was tested according to standards set by the International Standards Organization 9917-1. For evaluation of the setting time, each material was analyzed using Gilmore needles (456.5 g). Records were made when the needle failed to create a 1-mm deep indentation in three separate areas.

The injectability of PCL/ β -TCP composite paste was determined by pressing 2.5 g of as-prepared paste by hand through a 5-mL syringe with an opening diameter of 2.0 mm. This suggests that injection by hand has a slightly lower standard deviation than injection by machine with a preset load. After hydration at 37°C in 100% relative

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